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BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

^{19}F-NMR AND FLUORESCENCE POLARIZATION OF YEAST PLASMA MEMBRANE AND ISOLATED LIPIDS

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Summary: 16-Fluoropalmitic acid was incorporated biosynthetically into the plasma membrane of yeast and the F-NMR spectra of the intact membrane and of aqueous dispersions of membrane lipids were compared. The greater degree of resonance broadening in the intact membrane reveals a lesser degree of mobility of the acyl chains. Fluorescence polarization studies with a probe that monitors the polar environment and one that detects viscosity changes in the inner core of the bilayer indicate hindered motions of the polar head groups and acyl chains in the intact membrane as compared to liposomes prepared from membrane lipids. The observations indicate that the bulk of lipids in the yeast membrane experiences immobilization by proteins and that membrane proteins influence lipid -lipid interactions and inhibit lipid phase transition.

The generally accepted model of membrane structure implies that the bulk of lipids and proteins do not interact strongly and are independent of each other (1). This conclusion has been drawn from studies of mycoplasma membrane in which the intact membrane undergoes a phase transition in a temperature range very similar to that of aqueous dispersions of isolated lipids (2) and from experiments with erythrocyte membrane where digestion of 70% of membrane phospholipids has no detectable effect on the circular dichroism spectra of proteins (3). We reported recently that mobility of spin-labeled cysteine residues in plasma membrane of yeast Saccharomyces cerevisiae depends on membrane lipid composition (4). In the present report we

have employed ^{19}F-NMR and fluorescence polarization to monitor motions of the polar portion of the bilayer and those of the paraffinic chains in plasma membrane of this organism. Our studies reveal more hindered motion of the polar segment and greater viscosity in the inner core of the bilayer in the intact membrane than in aqueous dispersions of its isolated lipids. These observations indicate that there are strong interactions between proteins and the bulk of lipids in plasma membrane of yeast which involve both polar as well as nonpolar portions of lipids.

Materials and Methods

16-Fluoropalmitate was prepared as follows: To 30 ml of methanol containing 5% sulfuric acid were added 8 g of 16-hexadecanolide (Farchan Division, Storey Chemical Corporation). The mixture was refluxed for 2 hr., solvent was removed in a rotary evaporator and the product, 16-hydroxy methylpalmitate, was recrystallized from methanol. It was converted to 16-fluoromethylpalmitate by dissolving 6 mmoles of it into 5 ml of diethylamino sulfur trifluoride (5) and 5 ml of Freon II at 0°C. After 2 hrs. at room temperature the mixture was slowly added to 250 ml of an ice-water slurry. Methanol (100 ml) and 50% NaOH (3 ml) were added and the mixture was heated at 65°C for 5 hrs. After acidification with 20% HCl, it was extracted three times with 60 ml portions of methylene chloride, and dried over Na_2SO_4. The solvent was evaporated and 16-fluoropalmitic acid was recrystallized from acetone. Gas-liquid chromatography of the methylated product (4) showed a purity greater than 99%.

N-4-Nitrobenz-2-oxa-1,3-diazole-phosphatidylethanolamine (NBD-PE) was prepared according to Monti et al. (6). Fatty acids and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma Chemical Company. A strain of S. cerevisiae which requires both a saturated and an unsaturated fatty acid for growth (7) was grown under conditions described earlier (4) except that Tergitol NP-40 was substituted for Triton and palmitic acid was included in the medium at a concentration of 0.02%. Where used, the fluorinated fatty acid was included at a concentration of 0.01% and the concentration of palmitate was reduced to 0.01%. Cells were harvested in the logarithmic phase of growth and plasma membrane was purified as described previously (4). Lipid isolation procedures and fatty acid analysis have already been described (4). Aqueous dispersions of lipids (liposomes) were prepared according to Mandersloot et al. (8). For NMR spectroscopy yeast membrane was suspended in 0.05M Tris-HCl, pH 7.5, at a protein concentration of 40 mg/ml. With liposomes, the lipid concentration was approximately 20 mg/ml in the same

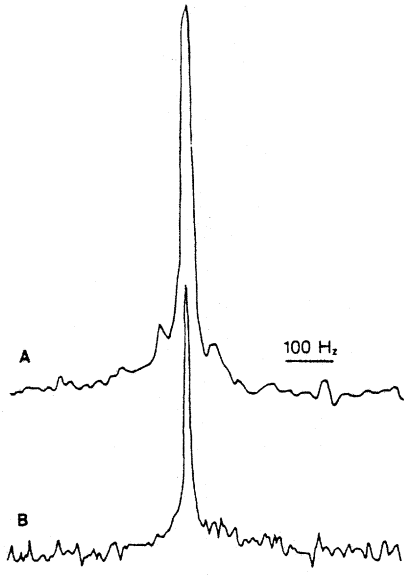

Figure 1. ^{19}F-NMR spectra of yeast plasma membrane (A) and its isolated lipids (B). Cells were grown in presence of 16-Fluoropalmitate.

buffer. Spectra of the samples, at 30°C, were recorded on a Brucker FT WH-90 NMR spectrometer operated at 90 MHz. Samples were labeled with the fluorescent probe as follows: To a dry film of 2 µg of NBD-PE or 0.5µg of DPH in a test tube 2ml of 0.05 Na phosphate buffer, pH 7.2, containing 0.25 mg membrane protein were added. After vortexing in the presence of a glass bead, fluorescence polarization was determined as described previously (9). Extracted lipids were labeled the same way except that the buffer was added to the dry film of the probe and 0.1 mg of lipids (equivalent to that of intact membrane). Fractionation of lipids (4) revealed that 16-fluoropalmitate was present only in the phospholipid fraction.

Results and Discussion

^{19}F-NMR: When the cells were grown in the presence of 16-fluoropalmitate, this fatty acid constituted 17% of the total plasma membrane phospholipid fatty acid composition. Spectra of the membrane and the isolated lipids are quite sharp and indicate a substantial degree of mobility in both systems (Fig. 1). Nonetheless, there is a greater degree of resonance broadening in the membrane than in the lipids such that the line-width (width of the line at half peak height) was 25 $\pm$ 2 Hz in the

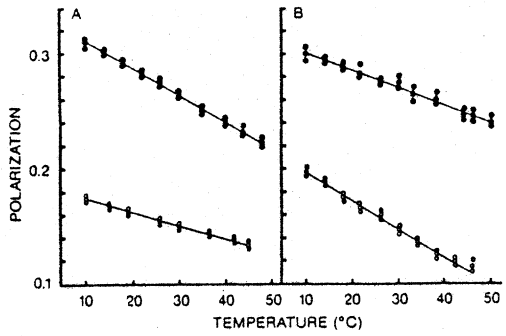

Figure 2. Fluorescence polarization of NBD-PE at different temperatures. A, cells supplemented with cis-C16:1; B, cells supplemented with trans-C16:1. (●), Membranes; (○) isolated lipids.

membrane compared to only 10 $\pm$ 2 Hz in lipids. Since fluorine is located on the terminal carbon of palmitate, the probe must be experiencing motion of the bilayer center. The result suggests that proteins must penetrate the lipid-bilayer in order to restrict motion of paraffinic chains in the inner core of the membrane. It can be argued that the difference in size and/or tumbling rate between membrane and lipid vesicles results in the observed differences in the resonance line broading. We, therefore, decided to also employ a different physical technique to eliminate this possibility.

Fluorescence Polarization: To monitor mobility in the polar environment (6), polarization of NBD-PE, incorporated into the membrane or lipids, was determined. Cells were enriched with palmitoleate (cis-C16:1) or palmitelaidate (trans-C16:1). No phase transitions were detected in plasma membranes enriched with either of these acids (Fig 2A &B). Furthermore, polarization of the probe in membranes enriched with palmitoleate was very similar to that of membranes enriched with palmitelaidate although these unsaturated fatty acids differ substantially in physical properties and each constituted more than 60% of the

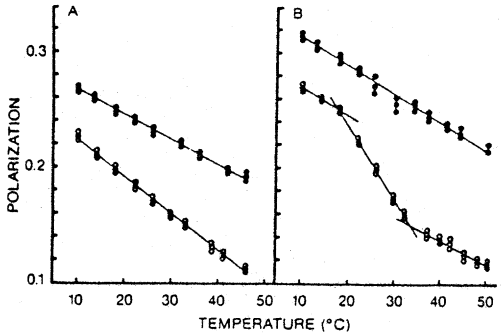

Figure 3. Fluorescence polarization of DPH at differnet temperatures. A, cells supplemented with cisC16:1; B, cells supplemented with transC16:1. (●), Membranes; (○), isolated lipids.

fatty acid content of phospholipids. Thus motion in the polar region of the bilayer appears to be independent of conformation and physical state of the paraffinic chains and of membrane microviscosity.

The polarization of NBD-PE in aqueous dispersions of isolated lipids was substantially lower than that of the probe in intact membrane. The values ranged from about 0.12 at 45°C to 0.20 at 10°C. The corresponding values for membrane were 0.22 and 0.3, respectively. Thus, in the two preparations containing different acids the probe experienced a greater degree of freedom of motion in isolated lipids than in the intact membrane. This indicates that there is an extensive lipid-protein interaction in the polar region of the bilayer leading to immobilization if the polar portions of phospholipids.

DPH is located in the interior of the lipid bilayer and the extent to which its fluorescence is polarized depends on microviscosity in this segment of the membrane (10). Polarization of DPH in the membrane enriched with palmitoleate in isolated lipids revealed no phase transitions (Fig 3A). Furthermore, polarization values were higher when the probe was intercalated into the membrane than when it was in liposomes.

The values ranged from 0.19 at 45°C to 0.27 at 10°C in the membrane with corresponding values of 0.11 and 0.22 in liposomes. The magnitude of the difference is significantly less than that found when NBD-PE was used (Fig 2). It can, therefore, be concluded that lipid-protein interactions are more extensive in the polar region of the bilayer.

In membranes enriched with palmitelaidate polarization values ranged from 0.22 at 45°C to 0.31 at 10°C (Fig 3B) which are higher than those obtained for palmitoeate-enriched membranes indicating a higher microviscosity in the former case. The polarization values of DPH in liposomes prepared from lipids of plasma membrane enriched with palmitelaidate were substantially lower than those of the probe in intact membrane and revealed a phase transition beginning at 18° and completing at 33°C (Fig 3B). The results suggest that proteins in the membrane restrict motion of paraffinic chains and impede chain-chain interactions.

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